

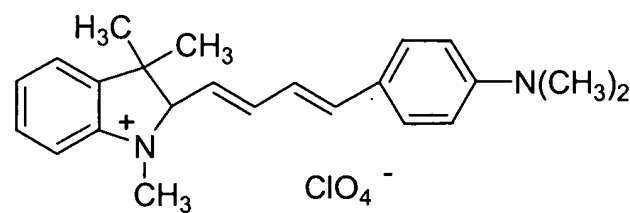
Listing of Claims:

1. (Currently Amended) A method of staining bacteria comprising: ~~working~~ adding a polymethine dye ~~on~~ to a sample in the presence of a substance capable of reducing nitrite ions to stain bacteria in the sample.

2. (Original) A method according to claim 1, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, aspartic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.

3. (Currently Amended) A method according to claim 1, wherein the polymethine dye is at least one selected from the following group consisting of:

(1) Thiazole Orange;



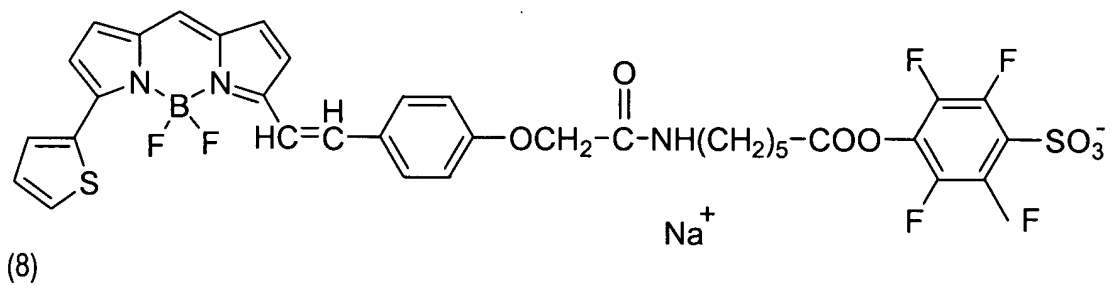
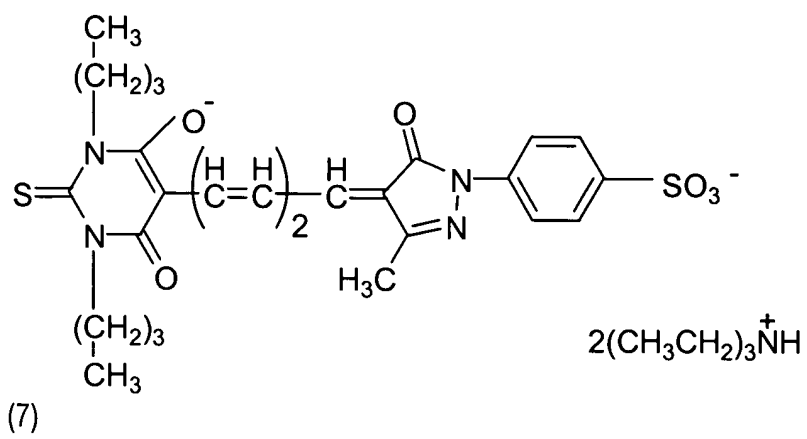
(2)

CN(CC1=CC=CC=C1S1C(=C/C=C/C=C/C2=CC=CC=C2N(C)C)C1)[Cl-][illegible]

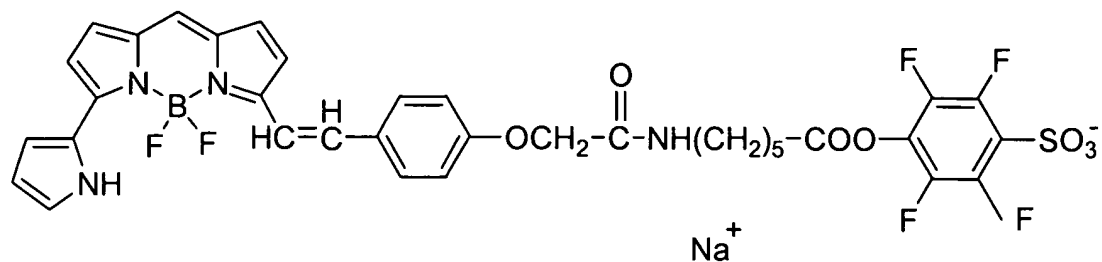
(5)

Chemical structure (5) shows a polymeric cationic species. It features two 1-methyl-2-benzothienyl groups connected to a central quaternary ammonium salt. The central part is a 4,4'-bipyridine derivative where the nitrogen atoms are quaternary ammonium cations, each with three methyl groups and a (CH₂)₃ chain. The chains are connected to the 2-positions of the bipyridine rings, which are also connected to the 2-positions of the benzothienyl groups. The overall charge is 4+ with 4 I⁻ counterions.

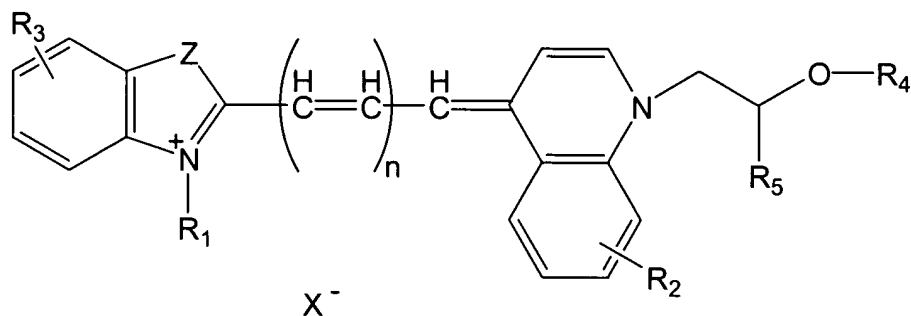
C[N+]1=C(S1c2ccccc2)C=CC=Cc3ccc4c(c3)ccc[n+](c4)C(C)C(C)[I-].[I-]



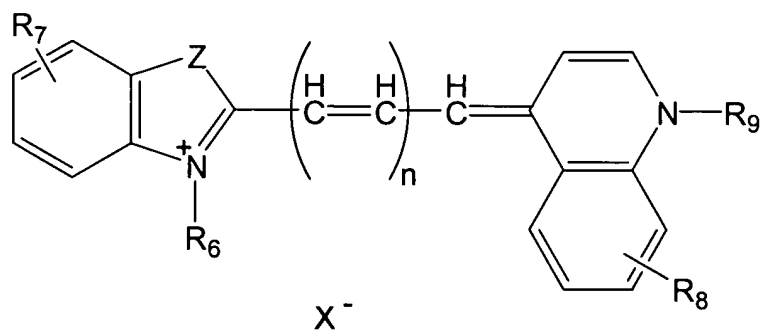
(9)



(10) a compound represented by the following general formula:



wherein R_1 is a hydrogen atom or a C_{1-3} alkyl group; R_2 and R_3 are a hydrogen atom, a C_{1-3} alkyl group or a C_{1-3} alkoxy group; R_4 is a hydrogen atom, an acyl group or a C_{1-3} alkyl group; R_5 is a hydrogen atom or a C_{1-3} alkyl group which may be substituted; Z is a sulfur atom, an oxygen atom or a carbon atom substituted with a C_{1-3} alkyl group; n is 1 or 2; X^- is an anion; and

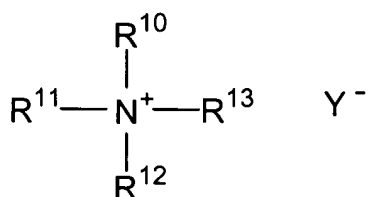


(11) a compound represented by the following general formula:

wherein R_4 R_6 is a hydrogen atom or a C₁₋₁₈ alkyl group; R_2 R_7 and R_3 R_8 are a hydrogen atom, a C₁₋₃ alkyl group or a C₁₋₃ alkoxy group; R_4 R_9 is a hydrogen atom, an acyl group or a C₁₋₁₈ alkyl group; Z is sulfur, oxygen or carbon having a C₁₋₃ alkyl group; n is 0, 1 or 2; ~~X~~ X^- is an anion.

4. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of a cationic surfactant.

5. (Currently Amended) A method according to claim 4, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein R^{10} is a C₆₋₁₈ alkyl group or (C₆H₅)-CH₂-; R^{11} , R^{12} and R^{13} , the same or different, are a C₁₋₃ alkyl group or a benzyl group; ~~Y~~ Y^- is a halogen ion.

6. (Original) A method according to claim 5, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

7. (Currently Amended) A method according to claim 1, wherein the ~~dye is worked under~~ sample is in an acidic state.

8. (Original) A method according to claim 7, wherein the acidic state is set at pH 2.0-4.5.

9. (Currently Amended) A method according to claim 4 Z, wherein a buffer of pKa 1-5.5 is used to maintain an acidic pH.

10. (Original) A method according to claim 9, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH, ϵ -aminocaproic acid-HCl, fumaric acid-HCl, β -alanine-NaOH and glycine-NaOH.

11. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of an inorganic salt of either sulfate or nitrate.

12. (Original) A method according to claim 1, wherein the dye is worked at 0.1 to 100 ppm in the sample.

13. (Currently Amended) A method according to claim 1, wherein the substance capable of reducing nitrite ions ~~exists~~ is present in the sample in such an amount that it can reduces the nitrite ions produced by bacteria of 10^5 to 10^8 /ml.

14. (Original) A method according to claim 1, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

15. (Original) A method according to claim 10, wherein the acid or the compound maintaining an acidic pH exists at 10 to 500 mM in the sample.

16. (Original) A method according to claim 1, wherein the sample is a urine, blood or spinal fluid.

17. (Currently Amended) A method of detecting and counting bacteria comprising the following steps of:

(1) ~~working~~ adding a polymethine dye ~~on to~~ to a sample by a method as described in ~~any one of claims~~ claim 1 to stain bacteria in the sample,

(2) introducing the ~~thus~~ treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and

(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the number of the bacteria.

18. (Original) A method according to claim 17, wherein the step (1) is carried out by the steps of

(a) mixing a sample with an aqueous solution containing a substance capable of reducing nitrite ions and/or a cationic surfactant to accelerate dye transmissivity of bacteria;

(b) staining the bacteria for a predetermined period with a polymethine dye;

19. (Original) A method according to claim 17, wherein the step (3) of discriminating and counting the bacteria is carried out in accordance with at least one selected from the following combinations of:

(i) a forward scattered light intensity and a forward scattered light pulse width;

(ii) a forward scattered light intensity and a fluorescent light intensity; and

(iii) a forward scattered light pulse width and a fluorescent light intensity.